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Microbiol. Immunol (1985) Vol 29 p. 959–972.
Lymphokines (N.Y.) (1984) vol. 9 p. 127–52.
J. of Immunology (U.S.) (1985) Vol. 134 p. 390 1–6
Molecular Immunology (1984) Vol. 21 No 12 p. 1229–36
Microbiology and Immunology (1986) Vol. 30 No. 4 p. 373–388.

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C3H
Selected US specifications from IPC sub-classes A61K
C12N C12P

(54) Monoclonal antibodies recognizing human interleukin-2-receptor

(57) Hybrid cell lines have been produced for the production of monoclonal antibody to an antigen found on activated human lymphocytes, the interleukin-2-receptor (iL-2-R) to the antibody so produced, and to therapeutic and diagnostic methods and compositions employing this antibody.

The antibody may be in the form of a chimeric animal-human antibody recognising iL-2-R wherein the F_c region is obtained from a human and the F_{AB} region is obtained from an animal.

GB 2 188 941 A

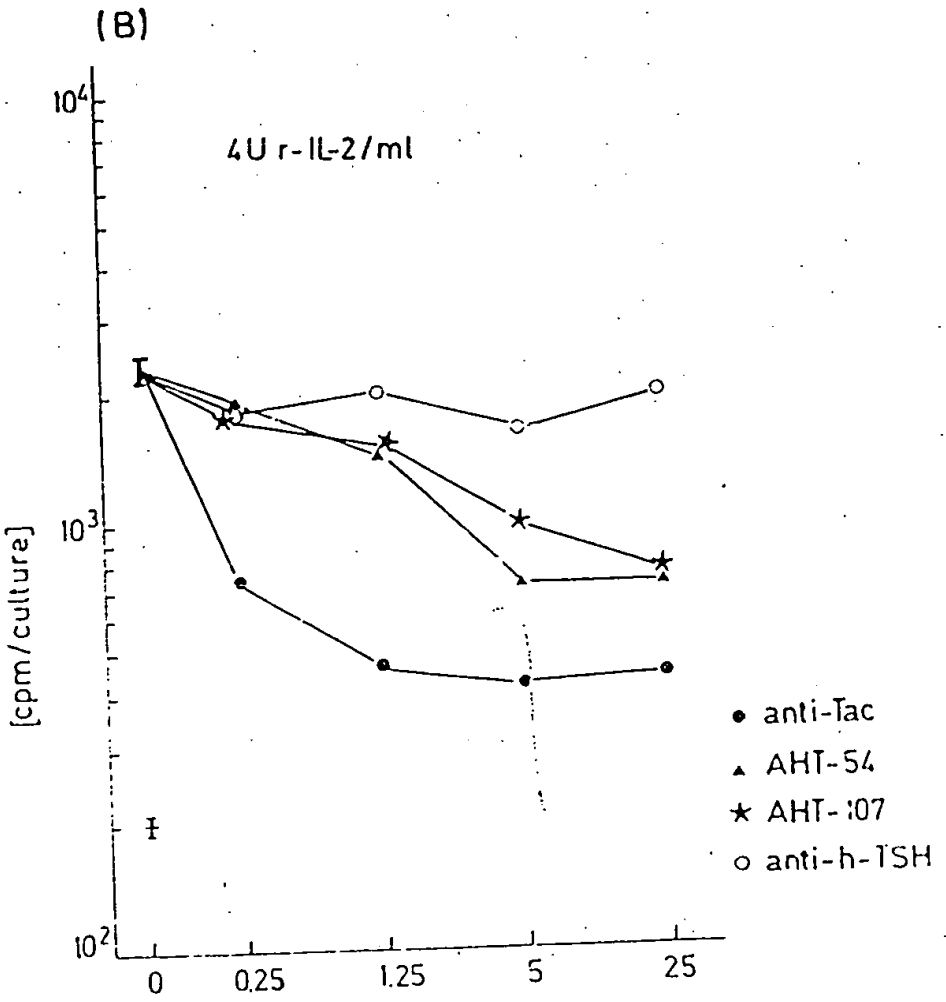

(B)
4U r-IL-2/ml
10^4
10^3
10^2
[cpm/culture]
0
0.25
1.25
5
25
anti-Tac
AHT-54
AHT-107
anti-h-TSH

FIG. 1a

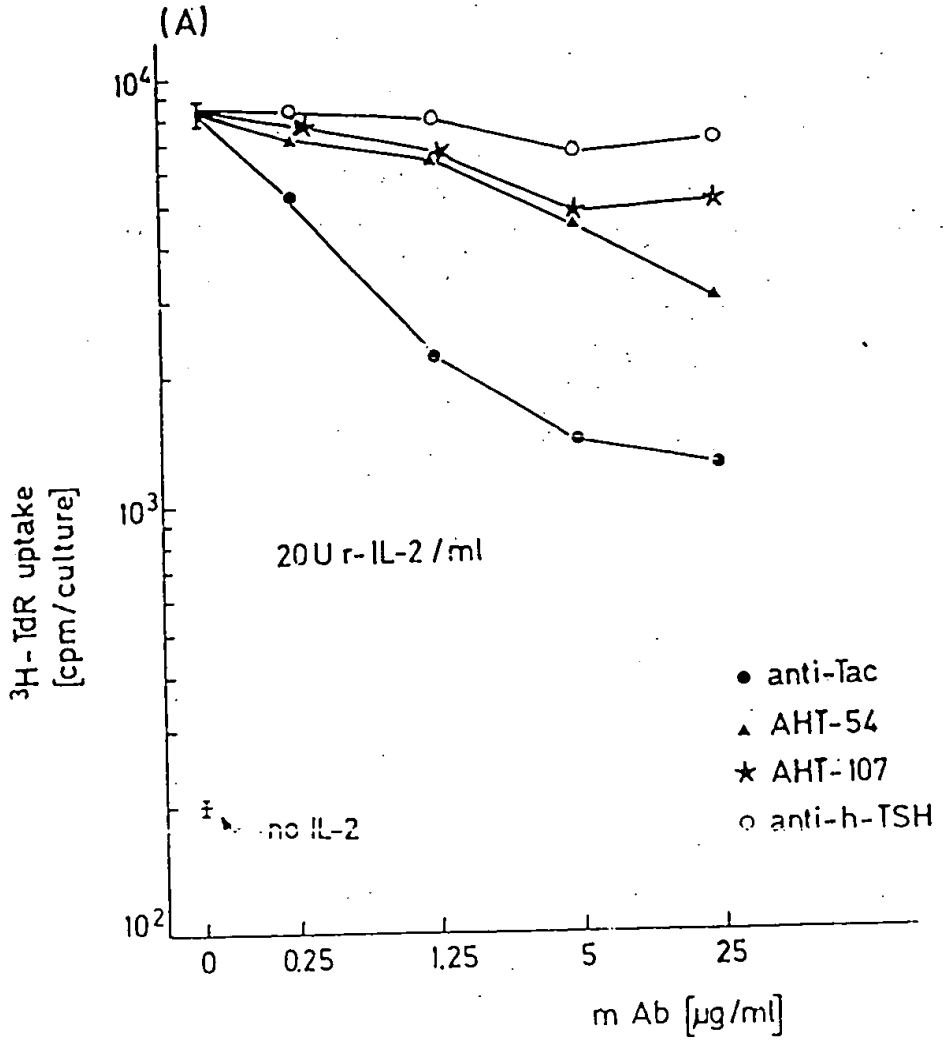

(A)
10^4
10^3
10^2
^{3}H-TdR uptake [cpm/culture]
20 U r-IL-2 / ml
no IL-2
anti-Tac
AHT-54
AHT-107
anti-h-TSH
0
0.25
1.25
5
25
m Ab [µg/ml]

FIG. 1b

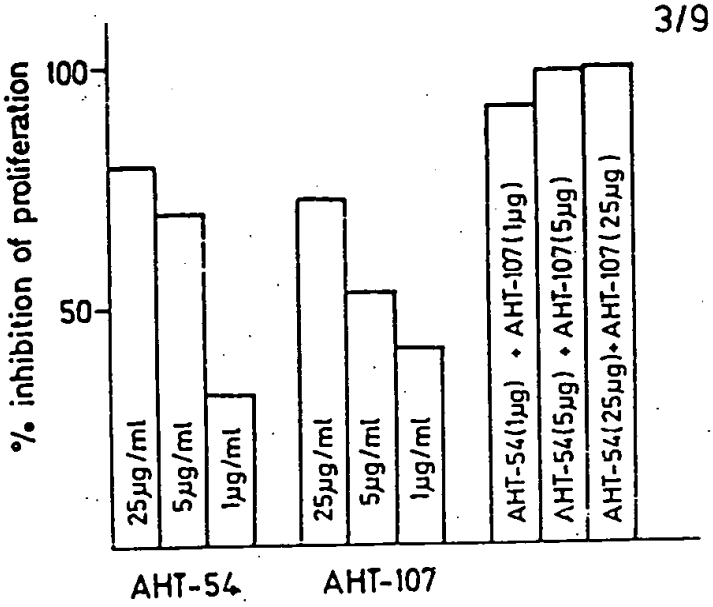

% inhibition of proliferation
100
50
25µg/ml
5µg/ml
1µg/ml
25µg/ml
5µg/ml
1µg/ml
AHT-54(1µg) + AHT-107(1µg)
AHT-54(5µg) + AHT-107(5µg)
AHT-54(25µg)+AHT-107(25µg)
AHT-54
AHT-107

FIG. 2

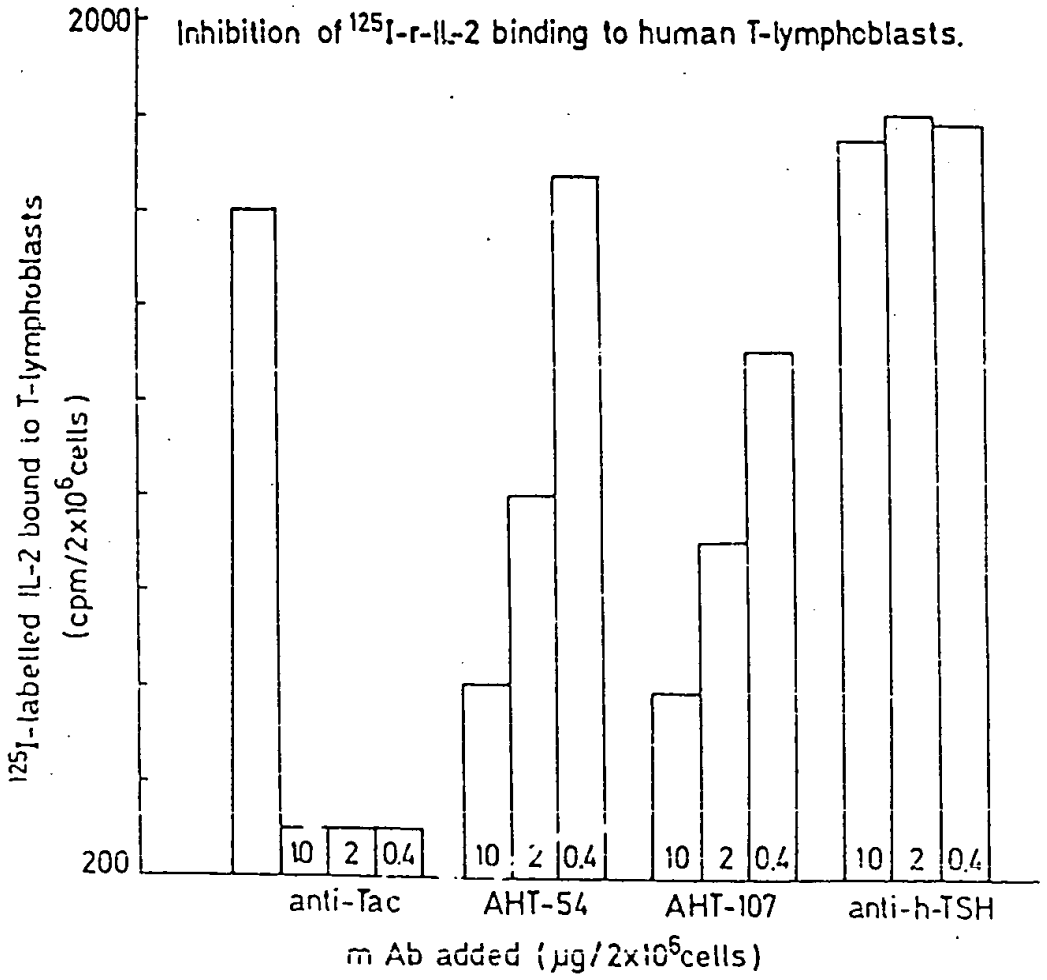

Inhibition of 125I-r-IL-2 binding to human T-lymphoblasts.
2000
200
125I-labelled IL-2 bound to T-lymphoblasts
(cpm/2x10^6cells)
10
2
0.4
anti-Tac
AHT-54
AHT-107
anti-h-TSH
m Ab added (µg/2x10^6cells)

FIG. 4

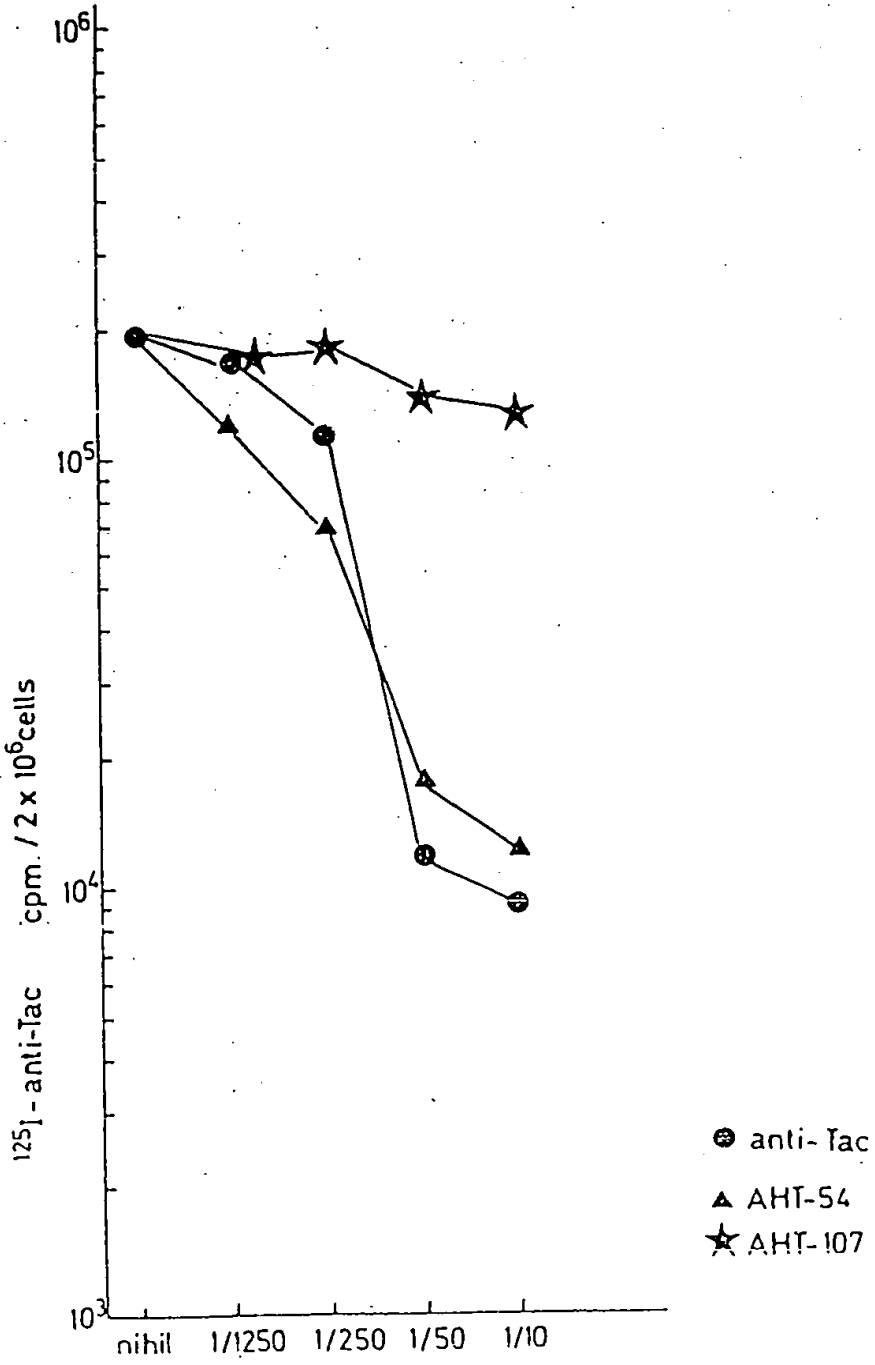

10^6
10^5
10^4
10^3
^{125}I - anti-Tac cpm. / 2 x 10^6cells
nihil
1/1250
1/250
1/50
1/10
anti-Tac
AHT-54
AHT-107

FIG. 3a

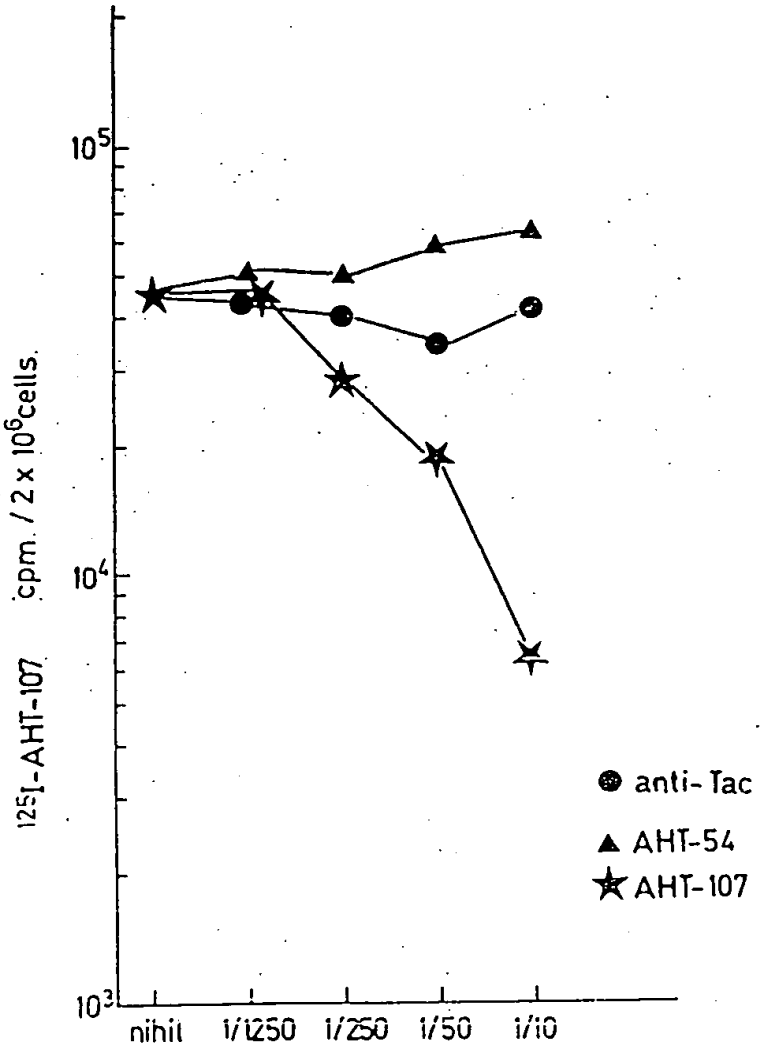

10^5
10^4
10^3
^{125}I-AHT-107 cpm. / 2 x 10^6cells.
nihil
1/1250
1/250
1/50
1/10
anti-Tac
AHT-54
AHT-107

FIG. 3b

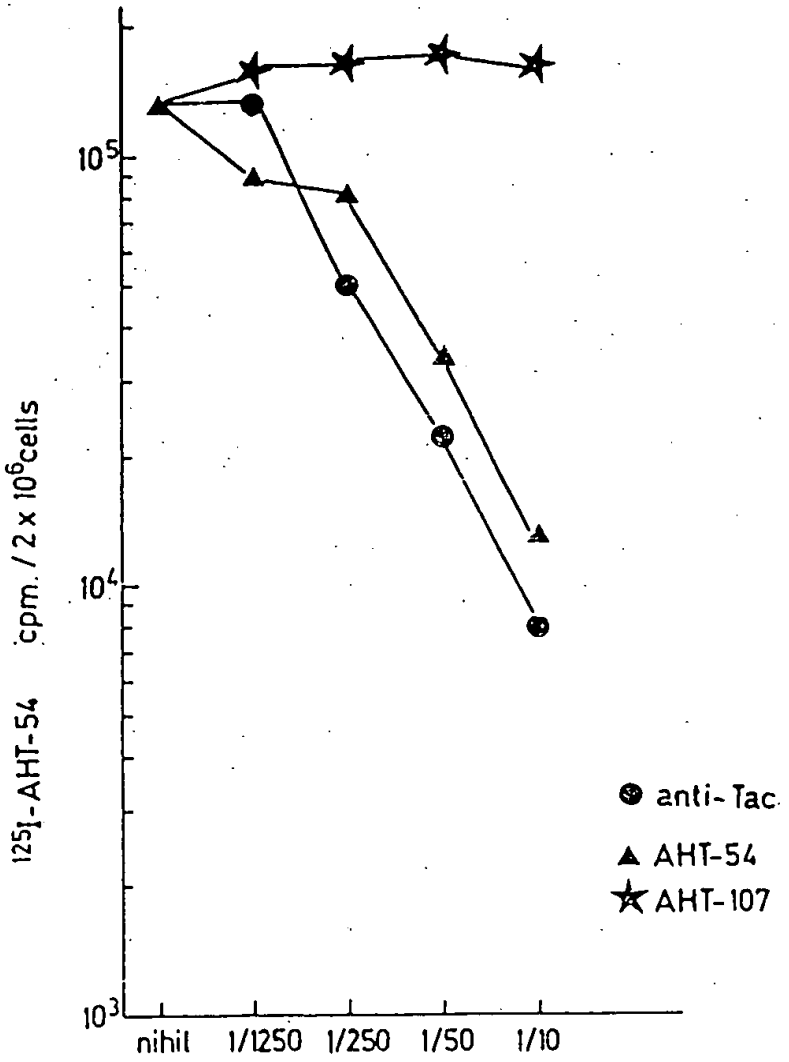

10^5
10^4
10^3
^{125}I-AHT-54 cpm. / 2 x 10^6cells
nihil
1/1250
1/250
1/50
1/10
anti-Tac
AHT-54
AHT-107

FIG. 3c

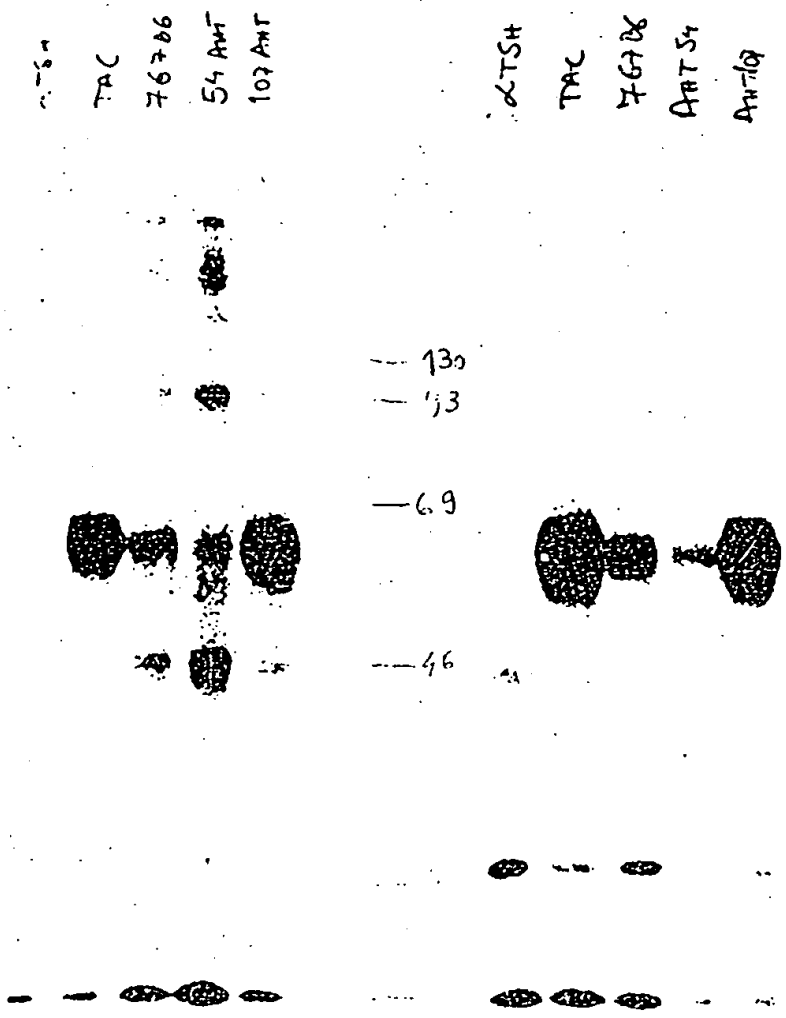


FIG. 5

FIG. 6

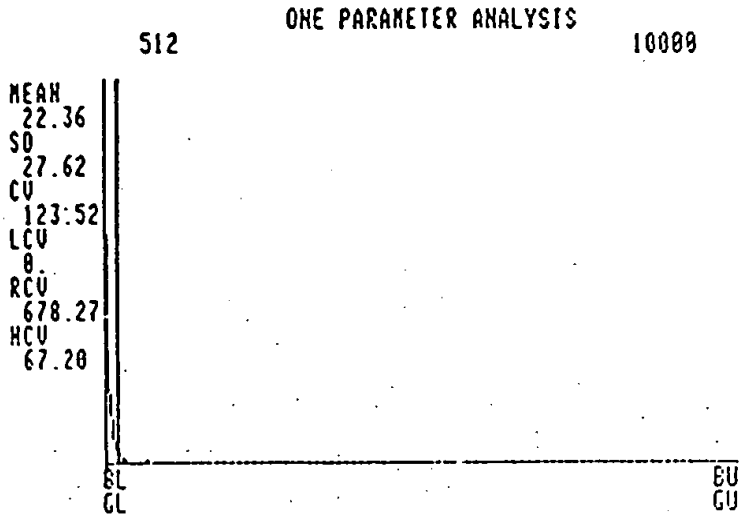


FIG. 7

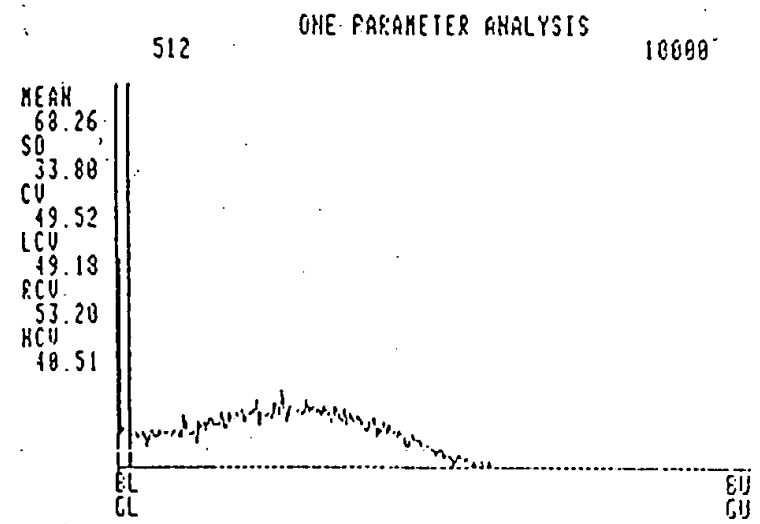


FIG. 8

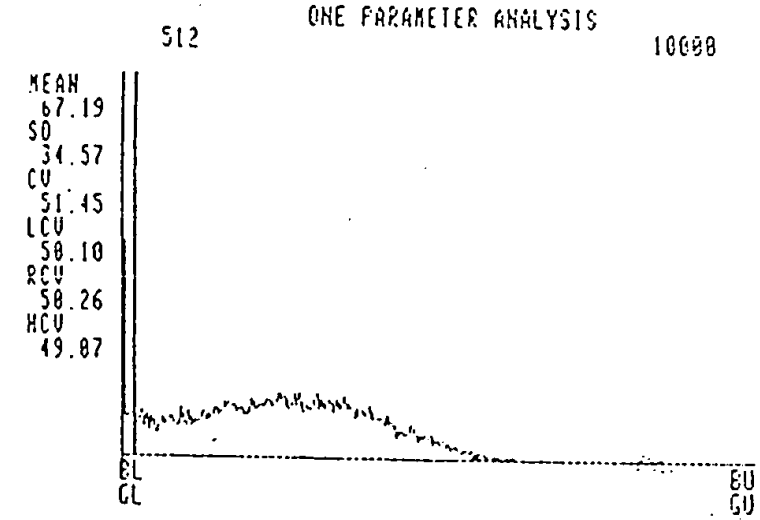


FIG. 9

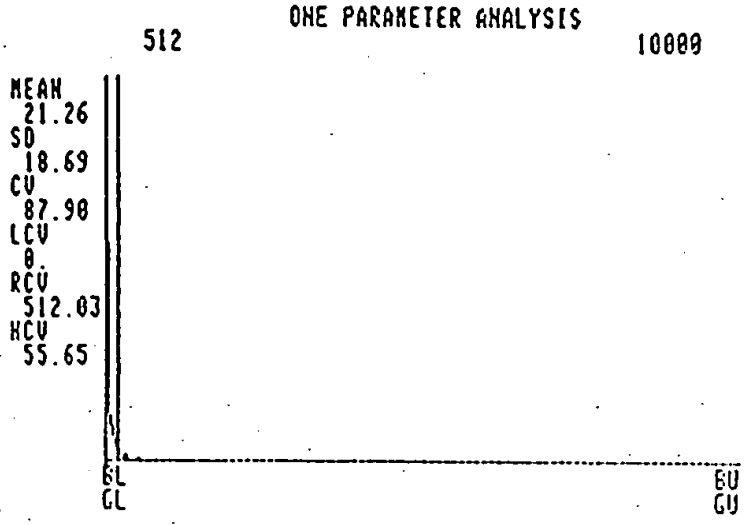


FIG. 10

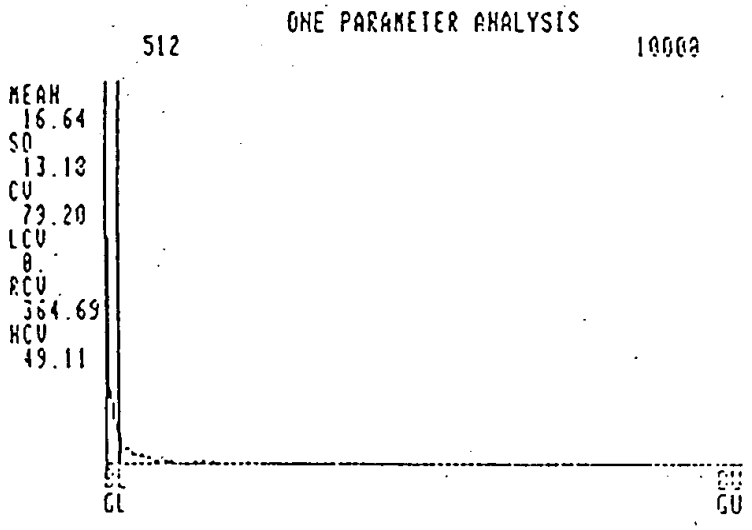
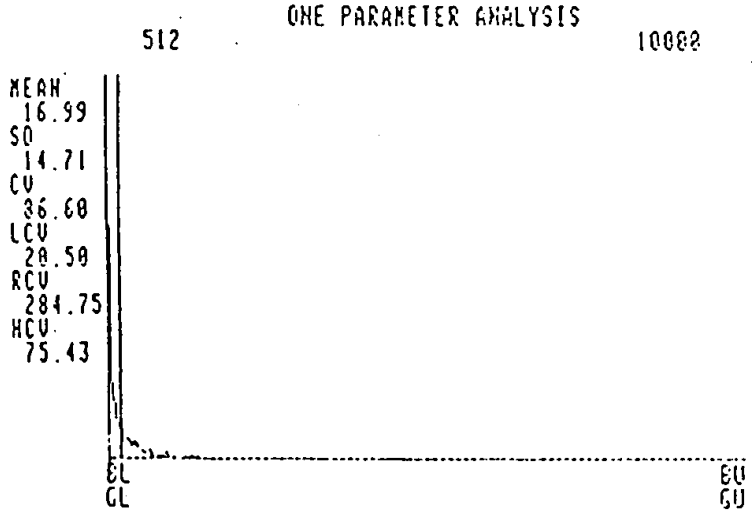


FIG. 11



SPECIFICATION

Monoclonal antibodies recognizing human interleukin-2-receptor

This invention relates generally to new hybrid cell lines and more specifically to hybrid cell lines for production of monoclonal antibody to an antigen found on activated human lymphocytes, the interleukin-2-receptor, to the antibody so produced, and to therapeutic and diagnostic methods and compositions employing this antibody.

INTRODUCTION

For many, if not all cells, the initial trigger for proliferation appears to be the interaction of growth factors with the cell surface growth factor receptor. Activation of the growth factor receptor leads in turn to yet undefined cytoplasmic signalling systems.

Resting T lymphocytes are long living cells in the G_0 phase of the cell cycle. They only enter proliferative cycles under antigenic stimulation in the presence of a T cell growth factor, interleukin 2 (IL-2). Receptors for IL-2 are not detectable on the surface of resting T cells. Expression of IL-2 receptors (IL-2R) is the consequence of interaction of antigen presenting cells with the antigen receptor. As shown recently, IL-2 receptor expression is a transient even and repeated restimulation by lectins (Cantrell, P.A., and K.A. Smith. (1984). Science (Wash. DC) 224:1312); (Osawa, H., and Diamantstein, T. (1984). J. Immunol. 132:2445) or the antigen (Reske-Kunz, A.B., D.v. Steldern, E. Rüde, H. Osawa and T. Diamantstein, (1984). J. Immunol. 133:1356) is required for continuous IL-2 receptor expression and consequently for long term cell growth.

Since, IL-2-R are expressed exclusively on activated lymphocytes monoclonal antibody (mAb) that reacts with the IL-2-R may be useful as specific and selective immunosuppressive agent. Furthermore, such antibodies may serve as diagnostic reagents in order to detect qualitatively and quantitatively activated lymphocytes as well as neoplastic cells expressing IL-2-R.

SUMMARY OF THE INVENTION

An object of the present invention is to provide a composition of at least two monoclonal antibodies recognizing human interleukin-2-receptor capable to inhibit interleukin 2 induced lymphocyte proliferation. In an preferred embodiment one or more of the antibodies are of the IgG_1 class. In another preferred emodiment the antibody composition is capable to inhibit interleukin-2 binding to the receptor. The present invention provides monoclonal antibodies of the IgG_1 class recognizing human interleukin-2-receptor capable to inhibit interleukin-2 binding to the receptor and in particular antibodies capable to inhibit interleukin-2 dependent lymphozyte proliferation. The antibodies of the present invention can be used for preparation of chimeric animal-human antibodies recognizing human interleukin-2-receptor wherein the constant F_c region of the immunoglobulin is obtained from human and the variance Fab region is obtained from an animal. Preferably the Fab region is obtained from mice.

The present invention further provides hybridoma cell lines, characterized by the production of monoclonal antibodies of the IgG_1 class recognizing human interleukin-2-receptor. Particular preferred are the hybridoma cell lines having the NTCC designation number... and.....

Human T-lymphoblasts expressing IL-2-R were prepared by known methods (Osawa, H., and Diamantstein, T. (1983) J. Immunol. 130:51.) were used to produce a mouse monoclonal antibody against IL-2-R by the technique of Köhler and Milstein (Köhler, G., and C. Milstein, (1975) Nature 256:495). The fusion resulted in two hybrid clones AHT-54 and AHT-107 producing anti-IL-2-R antibodies of IgG_1 subclass. The hybrid clones secreting anti-IL-2-R antibodies were selected as preferred embodiment of the present invention.

Both mAb i) inhibit binding of 125J labelled IL-2 to IL-2-R positive human lymphocytes, ii) inhibit IL-2 dependent proliferation in vitro and iii) precipitates the identical cell surface molecule of 55KD, the IL-2 binding protein. Competitive binding of AHT-54 and AHT-107 revealed that they recognize different epilops of the IL-2-R molecule.

AHT-107 is different from anti-Tac (Uchiyama, T. et al., (1981) J. Immunol. 126:1398) i) because competitive inhibition studies revealed that they recognize two different epitops of the IL-2-R molecule; AHT-107 is also different from 7G7 B6, a recently published antihuman IL-2-R mAb (A. Rubin, C. Kurman, E. Biddison, D. Goldman, and L. Nelson (1985) Hybridoma Vol. 4:91), because in contrast to 7G7 B6 AHT-107 inhibits binding of IL-2 to the IL-2-R as well as IL-2 dependent proliferation of lymphocytes.

Both mAb react specifically with activated lymphocyte (T and B) but not with resting lymphocytes or other non-lymphoid cells. This statement is based on FACS-analysis data (Fig. 6–11).

According to previous studies in animal models such Ab reacting with the rat (ART-18) and with the mouse IL-2-R (AMT-13 and M7/20) has been shown to inhibit selectively and specifically i) local GVH-reaction (Diamantstein, T. and H. Osawa, (1986), Immune Rev. 92 in press.) ii) cardiac allograft rejection (L. Kirkman, E. Kelley, A. Koltun, J. Schoen, A. Ythier and B. Strom, (1985), Transplantation 40:719), (L. Kirkman, L.V. Barrett, N. Gaulton, E. Kelley, A. Ythier and B. Strom,

(1985), J. Exp. Med. 162: 358.) and iii) T-cell-mediated autoimmune reaction such as acute autoimmune encephalomyelitis and adjuvant arthritis induced by T-cell transfer (Wekerle, H. and T. Diamantstein, (1986), Autoimmunity: Experimental and Clinical Aspects Eds: R.S. Schwarz, N.R. Rose. Ann. New York Acad. Sci., In press.)

The anti-IL-R monoclonal antibodies of the present invention are also useful as therapeutic agents in clinical syndromes which are associated with pathological proliferation of IL-2 dependent cells. Thus, for example, hyperimmune syndromes such as Host versus Graft(HvG) Graft versus Host (GvH) diseases and autoimmune diseases (e.g. multiples sclerosis, autoimmune diabetis, Crohn's disease) may be treated. In a preferred embodiment of the present invention, the anti-IL-2-R monoclonal antibodies are used as therapeutic agents directly without further modification thereof. Furthermore, the invention includes preparation of anti IL-2-R chimeric antibodies using human heavy chain of different classes and subclasses in combination with the variable region of the AHT-54 and AHT-107 mAb, in order to optimise for therapeutic use.

Alternatively, the antibodies may be coupled to drugs including cytotoxic agents. The monoclonal antibodies of the present invention are capable of recognizing specifically cells expressing IL-2 receptors, inhibiting their function and of eliminating them selectively.

The monoclonal antibodies of the present invention are also useful diagnostic reagents for cells which contain IL-2-R either on the cell surface or within the cells and in body fluids. Thus by means of the present invention, cells containing IL-2-R may be identified in samples having different kinds of cells. Localization of IL-2-containing cells is possible in cultured cell colonies or in tissue specimens. When used in this manner the monoclonal antibodies are preferably coupled to fluorescent, color-forming substances such as an enzyme or chromophor, or a radioactive substance (ELISA, RIA).

DETAILED DESCRIPTION OF THE INVENTION

The following description is intended to illustrate this invention without limiting the same in any manner especially with respect to substantially functional equivalents of hybridomas and monoclonal antibodies as described herein.

I. *Production of mAb*

Source of IL-2-R

IL-2-R expressing cells were prepared as described using human-T-lymphoblast. Mixed human peripheral blood lymphocytes were stimulated with 3 mg/ml of concanavalin A (Con A) for 3 days. The cells were converted, treated with α-methyl mannoside (20 mg per ml), washed and used as immunogens in culture medium. Cultures were performed in Click's RPMI medium (Seromed GmbH, München, F.R.G.) supplemented with 2×10^{-3} M L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 100 U ml^{-1} penicillin, 100μg ml^{-1} streptomycin, and 5 to 10% (v/v) fetal calf serum (FCS; batch No. 104; Seromed GmbH).

Immunization, cell fusion, cloning, and production of monoclonal antibodies (mAb)

Ten-week-old BALB/c mice were primed with 2×10^7 T lymphoblasts. The cells were injected in 0,1 ml portions (10^6 cells) subcutaneously into the footpads and into the necks of the mice as well as i.V. (10^7 cells in 0.5 ml). Four weeks later, the mice were challenged i.v. with 10^7 T-lymphoblasts. Three days later, spleen cells from the immunized mice were fused with X63-Ag8.653 mouse myeloma cells in the presence of polyethylene glycol (Köhler and Milstein, (1975), Nature 256:495, as modified by Lemke H., G.J. Hämmerling, C. Höhmann and K. Rajewsky, (1978), Nature 271:249). Fused cells suspended in HAT medium were distributed into each well of ten 24-well tissue culture plates (1 to 2×10^6 spleen cells/well). Supernatants of the wells in which vigorous growth was observed after 3 to 4 wk were screened for their capacity to bind a) human T lymphoblasts, b) mouse T lymphoblasts, and c) human thymocytes attached to the surface of the wells of microtiter plates. Cell-bound immunoglobulin was then detected by enzyme-linked immunosorbent assay (ELISA) as described (Kincade, P.W., G. Lee, L. Sun, and T. Watanabe, (1981), J. Immunol. Methods 42:17.) by using β-galactosidase-coupled sheep $F(ab')_2$ anti-mouse immunoglobulin (New England Nuclear, Dreieich, F.R.G.) as a second antibody. The hybridomas grown in HAT or RPMI medium that constantly produced antibodies binding specifically to human T lymphoblasts were selected. Supernatants of growing hybridomas were repeatedly tested and selected for hybridomas producing supernatants active in the functional assay (inhibition of the T-lymphoblast response to IL-2) as well as the absorption assay (inhibition of the capacity of T lymphoblasts to absorb IL 2 after preincubation). Positive hybridomas were cloned by limiting dilution with mouse thymocytes used as a feeder layer. The clones were retested and expanded. The supernatants of the relevant clones were used for isolation and purification of the mAb.

Purification of the mAb

As tested in the Ouchterlongy double immunodiffusion test with rabbit anti-mouse IgM, IgA, IgG1, IgG2a, IgG2b, and IgG_3 sera (Miles Laboratories, Ltd., Slough, England), the hybridoma clone AHT-54 and AHT-107 were found to produce IgG1 antibodies. Excepting the initial screening experiments, in which unpurified culture supernatants were used, the

following experiments were performed with purified IgG1. Purification was achieved by successive binding/elution from protein A-Sepharose (Pharmacia Fine Chemicals) according to the method described by Ey et al, (Ey, P.L., S.J. Prowse and C.R. Jenkin, (1978), Immunochemistry 15:429). About 600 ml of the culture supernatants, brought to pH 8.0, were passed over a 5 ml protein A-Sepharose column equilibrated in 0.1 M sodium phosphate buffer (pH 8.0) IgG1 was eluted from the column with 0.1 M sodium citrate buffer (pH 6.0). The purified antibody was then dialyzed against a buffer containing 0.01 M HEPES (pH 7.4) and 0.9% NaCl. The purity of mAb was confirmed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis performed in reducing conditions as described (Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227:429). The protein concentration of the purified IgG1 was determined by absorption of ultaviolet light at 280 nm, assuming an extinction coeficient (1% w/v:1cm) of 14, and by the method of Lowry et al, 1951 (Lowry, O.H., NJ. Rosebrogh, A.L. Farr, and R.J. Randall (1951). J. Biol. Chem. 193:265.) with bovine serum albumin (BSA) used as the standard.

Recombinant Interleukin-2 provided from Sanolez Mena was used. ^{125} I-labelled recombinant IL-2 was produced from NEN.

Labelling of the mAB with ^{125}J

MoAb were labelled with ^{125}J according to McConahey and Dixon (McConahey, P.J., and F.J. Dixon, (1980) Methods Enzymol, 70:210). Briefly, 20 μg of IgG1 dissolved in 50 μl of Na^{125}J(100 mCi ml^{-1}, carrier-free; Amersham Buchler). Ten microliters of chloramine-T (2.5 mg ml^{-1} in 0.05 M Na-P) were added to the mixture. After 45 sec of incubation at room temperature, 20 μl of $Na_2S_5O_5$ (3 mg ml^{-1} in 0.05 Na-P) were added to the tube. The mixture was immediately loaded onto a 15-ml Sephadex G-75 column (prewashed with 0.05 M Na-P containing 4% BSA and washed consecutively with 0.05 M Na-P until the eluate was protein-free), and the radiolabel in the excluded fraction was collected.

Detailed description of the drawings:

Figure 1

Inhibition of IL-2 dependent human T-lymphoblast proliferation by different mAbs.

2×10^6 human T-lymphoblasts were incubated for 3 days in 0.2 ml of medium containing the indicated amounts of recombinant IL-2 (Fig. 1a, 20U/ml; Fib. 1b,4U/ml) in the absence or presence of the different mAbs, anti-Tac (–O–), HT-54 (–Δ–), AHT-107 (–☆–) and of a control mAb anti-human-TSH (–O–). The cells were pulsed with ^{3}H-thymidina for the last 4 h of the incubated period. Incorporation of ^{3}H-thymidine was measured according to the standard procedure (Diamantstein et al, Mol. Immunol., (1984), 21:1229.

Figure 2

Synergistic action of AHT-54 and AHT-107 mAbs on IL-2 dependent proliferation

T-lymphoblasts were cultured with 20U/ml of r-IL-2 for 3 days (for detail see Fig. 1) in the presence of either AHT-54 or AHT-107 mAB or in combination of both mAbs.

Figure 3

Competition for the binding of ^{125} I-labelled antibodies

2×10^6 human T blasts were first suspended in 100 μl of a binding buffer (PBS=0.5% BSA/10 mM NaN_3) containing different dilutions of the mAbs anti-Tac (–O–), AHT-54 (–Δ–) and AHT-107 (–☆–). The suspensions were mixed with 100 μl of a 1:40 dilution of the ^{125}I-labelled mAb.

The mixture was incubated for 1h at 4°C. The relative amount of ^{125} I-labelled mAb (cpm) bound to the pelleted cells was measured by using a gamma-radiation counter after washing them twice with the binding buffer.

Figure 4

Inhibition of ^{125}I-IL-2 binding to human T blasts by different mAbs

2×10^5 human T blasts were first incubated for 30 min. at 37°C in 0.25 ml of a buffer (RPMI/Hepes/BSA/NaN_3) containing the indicated amounts of different mAbs. The incubation was further continued at 37°C in the presence of ^{125}I-IL-2. After 40 min. the incubation mixture was centrifuged to pellet the cells and the pelleted cells were taken up in 100 μl of the buffer and overlaid on the oil phase consisting of dibutylphthalate/olive oil (10+3). After centrifugation the tips of the tube containing the cell pellets were cut out and counted in a gamma-radiation counter.

Figure 5

SDS-PAGE analysis of immunoprecipitates with different mAbs

2×10^7 human T blasts were surface-iodinated with 0.5 mCi of Na [^{125}I] and lysed in 0.5 ml of the lysis buffer. The lysate was centrifuged and preabsorbed with 1/5 volume of protein A-sepharose beads (10 μl) through a bridge antibody rabbit anti-mouse IgG. After 1h at 4°C the beads were washed three times with a buffer containing 50mM Tris-HCL, pH 8.3, 450 mM NaCl, 5mM KI, 0.02% NaN_3 and 0.5 Nonidet P-40, and extracted with 100 μl of the sample buffer. 50 μl aliquots of the extracts were subjected to SDS-PAGE analysis either under non-reducing (lanes 1–6) or reducing (lanes 7–12) conditions. the mAbs used were control mouse UPC-10 ascites (lanes 1 & 7), anti Tac ascites (lanes 2 & 8),

AHT-54 ascites lanes 4 & 10), AHT-107 ascites (lanes 5 & 11) and AHT-107 culture supernatants (lanes 6 & 12).

Figures 6 to 11
FACS-analysis

Human peripheral blood cells (HPBL) and activated T-lymphoblasts derived from HPL were incubated at 4°C in presence of 0.1 % NaN_3 for 30 min with AHT-54 or AHT-107 mAB (1:1000 ascites fluid and as negative control with a TSH, washed and stained using saturating amount of goat anti-mouse IgG labelled with FITC. Fluorescence activated cell sorter analysis performed with Epics V.

Fig. 6 shows the negative control. The α TSH antibody did not bind to the T-lymphoblasts. In Fig. 7 and 8 it is shown that AHT 54 (Fig. 7) and AHT 107 (Fig. 8) are capable to bind to the lymphoblasts. Fig. 9 to 11 are refering to the same experiment except the lymphoblasts are substituted by HPBL. With none of the three antibodies a reaction occurs.

CLAIMS

1. A composition of at least two monoclonal antibodies regognizing human interleukin-2-receptor capable to inhibit interleukin-2 dependent lymphocyte proliferation.

2. A composition according to claim 1, wherein at least one of the monoclonal antibodies is of the IgG_1 class.

3. A composition according to any of claims 1 and 2 inhibiting interleukin-2 binding to the receptor.

4. A monoclonal antibody of the IgG_1 class recognizing human interleukin-2-receptor capable to inhibit interleukin-2 binding to the receptor.

5. A monoclonal antibody according to claim 4 capable to inhibit interleukin-2 induced lymphozyte proliferation.

6. A chimeric animal-human antibody recognizing human interleukin-2-receptor.

7. A chimeric antibody according to claim 6, wherein the F_c region is obtained from human and the Fab region is obtained from an animal.

8. A chimeric antibody according to any of claims 6 and 7, wherein the Fab region is obtained from mice.

9. Hybridoma cell lines, characterized by the production of monoclonal antibodies of the IgG_1 class recognizing human interleukin-2-receptor.

10. The hybridoma cell lines of claim 9 having the NTCC designation number . . . and . . . . .

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